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Antituberculosis Activity Of *Barlaria Buxifolia Linn* Using Microplate Almar Blue Assay (MABA)

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ABSTRACT

Multidrug-resistant *Mycobacterium tuberculosis* strains have rapidly become a global health concern. Medicinal plants offer a hope for developing alternate medicines for the treatment of TB. Natural products have been used worldwide in traditional medicine for the treatment of various diseases. Ayurveda system of medicine uses a number of plants to treat pulmonary tuberculosis. In the present pilot study evaluation of antimycobacterial activity against *M. tuberculosis* H37Rv using the microplate almar bluedye assay has been carried out. Extracts of roots, stem and leaves of the *Barlaria buxifolia* Linn from the family Acanthacea, demonstrated significant antimycobacterial activity. This study shows that ethanol extract of the stem and leaves was found to be active at minimum inhibitory concentrations (MIC) of 25 and 50µg/ml and provides potential for the development of urgently needed novel antituberculous therapeutics.

Keywords: Antituberculous, *Mycobacterium tuberculosis*, *Barlaria buxifolia* Linn, Antimycobacterial activity, Microplate Almar Blue Assay (MABA).

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INTRODUCTION

Tuberculosis (TB) is a contagious-infectious disease mainly caused by *Mycobacterium tuberculosis,* which is an aerobic pathogenic bacterium that establishes its infection usually on the lungs [1]. About one third of the world's population including 40 percent from India is estimated to be infected with it [2]. The rising incidence in mortality rates of tuberculosis is attributable to co infection with HIV in endemic areas and the spread of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) [3]. MDR-TB was documented in nearly every country surveyed from 1994 to 2000 by the World Health Organization-International Union against Tuberculosis and Lung Disease as part of the Global Drug Resistance Surveillance Project [4].

The situation with multidrug resistant (MDR) tuberculosis (TB) today worries the health authorities of the whole world, mainly in the developing countries, where the situation is more severe. The increasing incidence of MDR-TB worldwide highlights the urgent need to search for newer anti-tuberculosis compounds/ drugs. Therefore, the present study was carried out to check the antibacterial activity of different solvent extracts of this plant against M. tuberculosis. A number of antimicrobial agents already exist for various purposes but the search for new antimicrobial agents should be a continuous one since the targets microorganisms often evolve into new genetic variants which subsequently become resistant to existing agents.

The appearance of resistant strains to the available medicines in market makes urgent search for new synthetic or natural tuberculostatic drugs. The primary weapons in the treatment of TB are rifampicin, isoniazid, pyrazinamide and also the discovery of streptomycin [5-8], which are collectively called the 'frontline' drugs. When these fail the patients are treated with second line drugs. In addition, most of the second-line drugs are unaffordable to the common man and have serious side effects.

With the current trend in the biotechnology of plant tissue culture [9], it would appear that man may soon have to depend on plants as a source of a number of antimicrobial agents since these plants will most likely continue to produce antimicrobial agents which could be used against infections by microorganisms.

Natural products are proven templates for the development of new scaffolds of drugs [10-12].They have received considerable attention as potential anti-TB agents [13]. Natural products have been used worldwide in traditional medicine for the treatment of various diseases. An approach to the search for new drugs is to look in nature, mainly for the extremely rich and varied flora of the tropical areas. In this search various reasons have been put forward to explain the success of natural products in drug discovery; their high chemical diversity, the effects of evolutionary pressure to create biologically active molecules, the structural similarity of protein targets across many species, and so on [14].

India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for curing various diseases [15, 16]. Medicinal plants offer a great hope to fulfill these needs and have been used for curing

diseases for many centuries. These have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties [17]. Plant extracts are attractive sources of new drugs and is the state of art process to identify the active compounds contained in crude natural products. Plants belonging to Acanthaceae family (*Adhatoda vasica*) already reported to have anti-tuberculosis activity [18, 19]. Therefore, the present study is to evaluate by MABA, the potential antitubercular activity of parts of *Barlaria buxifolia* Linn. No record investigation has been carried out on phytochemical screening and medicinal usage of any parts of *B.buxifolia*. However, some other members of the family that are related to *B.buxifolia* have been explored to some pharmacological activities.

MATERIAL AND METHODS

Plant Collection

Barlaria buxifolia was collected from scrubby jungles of Nelamangala and Magadi, Bangalore Rural District in May-June 2010. The plant was authenticated by Dr. Krishnegowda, Director, Dayananda Sagar College of Biological Sciences, Bangalore.

Preparation of Extracts

The plant material was washed thoroughly in running tap water to remove soil particles and air dried at room temperature (40^{o} C) for few days. The roots were separated out, stem and leaves were chopped into small parts in a blender and pulverized to a fine powder using a mixer grinder and stored in airtight bottles. Approximately 1000g of the pulverized plant parts of roots and leaves were extracted successively with carbon tetrachloride and stem was extracted with carbon tetrachloride, ethyl acetate, ethanol and methanol in a soxhlet extractor. The solvent was removed under reduced pressure at 30^{o} C with a rotary evaporator to obtain the extracts as solid residues. All the dried extracts were stored at -20^{o} C until used.

Antitubercular Activity Assay

The antitubercular activity of carbon tetrachloride extracts of roots and leaves of *B. buxifolia* and carbon tetrachloride, ethyl acetate, ethanol and methanol extracts of stem were assessed against *M.tuberculosis* using microplate Almar Blue Assay (MABA) [20]. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the middlebrook 7H9 broth and serial dilutions of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with paraffin and incubated at 37^{o} C statistically for five days. After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% Tweeen 80 was added to the plate and incubated for 24 hours. A blue color in the well was interpreted as no bacterial growth and pink colored was

scored as growth. The MIC was defined as the lowest drug concentration which prevented the color change from blue to pink.

Radiometric

BACTEC 460^{R} instrument is a semi-automated radiometric drug susceptility testing (RAD) method that measured the $^{14}CO_2$ produced by metabolic break down of (1-^{14}C) palmitic acid in a liquid middlebrook 7H12 medium [17, 18, 19]. With this method, multiple concentrations can be tested and an MIC calculated [20]. However, although results are generally available in 5 days, the tests performed on the BACTEC system are costly and are not suited to the evaluation of large numbers of compounds [21].

RESULTS AND DISCUSSION

Carbon tetrachloride, ethyl acetate, ethanol and methanol fractions after successive extraction of roots, stem and leaves of *B.buxifolia* inhibited the growth of *M.tuberculosis* H37Rv ATCC 27294 sensitive strain at MIC values 25 and 50µg/ml respectively (Table 1). The tested extracts inhibited the growth of bacteria at different MIC values. Among the six extracts, ethanol and methanol extracts of stem showed the significant activity at 25 and 50µg/ml, whereas carbon tetrachloride extract of leaves and carbon tetrachloride and ethyl acetate extracts of stem showed activity at 50 and 100µg/ml. Although the carbon tetrachloride extract of root showed activity >100µg/ml. We, for the first time showed that a more refined fraction of the extract is active against *M.tuberculosis*. *Adhatoda vasica* of Acantheceae family has been reported to have antimyobacterial properties. However the activity in *B.buxifolia* of Acanthaceae has not been reported so far.

In this we have tested the extracts at a concentration of 100µg/ml. Now we are trying to purify the active anti TB principles from the extracts using techniques like Preparative TLC, Column chromatography, HPLC. Since carbon tetrachloride, ethyl acetate, ethanol and methanol extracts of stem and leaves showed activity at different concentrations. It is possible that the active compounds could be flavonoids, alkaloids and phenolic compounds. Once purified these compounds are expected to exert their anti TB activity at much lower concentration.

Table –1. Antitubercular activity of *Barlari buxifolia* Linn. Plant extracts tested against *Mycobacterium tuberculosis*. Activity was assessed using the microplate Almar Blue Assay (MABA) after 5 days of treatment with the extracts.

Plant	Part Used	Extract	MIC µg/ml
Barlaria buxifolia	**Stem**	Carbon tetrachloride	100
		Ethyl acetate	50
		Ethanol	25
		Methanol	50
	Leaves	Carbon tetrachloride	50
	Root	Carbon tetrachloride	>100

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CONCLUSION

With increasing rates of tuberculosis worldwide and the rise of MDR-TB and XDR-TB, there is a need for novel antituberculosis agents. Using the MABA as a screening tool this study assessed antimycobacterial properties of extracts of medicinal plants. This study serves to validate traditional knowledge and adds to the growing literature on botanical sources identified as providing important novel antituberculosis compounds.

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